April 9, 2001

CENTER FOR VETERINARY BIOLOGICS NOTICE NO. 01-02

Subject: Issuance of New or Revised Supplemental Assay Methods

To: Biologics Licensees, Permittees, and Applicants
Veterinary Services Management Team
Directors, Center for Veterinary Biologics

The following Supplemental Assay Methods (SAMs) have been approved:

MVSAM0105.01 Supplemental Assay Method for the Titration of Infectious Bovine Rhinotracheitis Virus in Vaccines. This SAM was rewritten to meet the current NVSL/CVB QA requirements, to clarify practices currently in use in the CVB-L, and to provide additional detail. No significant changes were made from the previous protocol. This document supersedes the May 1, 1982, version.

MVSAM0108.01 Supplemental Assay Method for the Detection of Extraneous Bovine Viral Diarrhea Virus in Modified-Live Vaccines. This document supersedes the November 1, 1987, version. This SAM was rewritten to meet the current NVSL/CVB QA requirements, to clarify practices currently in use in the Center for Veterinary Biologics-Laboratory, to provide additional detail and to reflect these changes from the superseded protocol:

1) replacement of simultaneous inoculation of bovine embryonic kidney (BEK) cells by inoculation of bovine turbinate (BT) cell monolayers,

2) inclusion of BVDV type II Reference Virus for a Positive Control,

3) inclusion of newly added vaccine viruses to the serum neutralization step,

4) inclusion of two passages in 25-cm^2 flasks prior to inoculation of Lab-Tek® Slides,

5) inclusion of a direct fluorescent antibody technique (DFAT) for detection of BVDV type I and II.

6) expanding the scope to allow testing of all modified live vaccines that are grown on cell lines that are permissive for BVDV.

MVSAM0116.01 Supplemental Assay Method for the Phenotypic Examination of Pseudorabies Virus for Thymidine Kinase Activity by a Plaque Selection Method. This document supersedes the November 1, 1987, version. This SAM was rewritten to meet

the current NVSL/CVB QA requirements, to clarify practices currently in use in the CVB-L, and to provide additional detail. The following is a list of significant changes made from the superseded protocol:

8.1 The size of the tissue culture flask was changed to 25 cm^2 from 75 cm^2, and the media addition was adjusted accordingly.

8.2 The final passage in Madin-Darby bovine kidney (MDBK) cells was changed to 25 cm^2 from 96-well plates.

8.3 The media formulations were updated.

8.4 The validity requirement for a predetermined titer for the PRV Positive Control was removed.

MVSAM0121.01 Supplemental Assay Method for Titration of Porcine Rotavirus in Modified-live Vaccines. This SAM was rewritten to meet the current NVSL/CVB QA requirements, to clarify practices currently in use in the CVB-L, and to provide additional detail. No significant changes were made from the previous protocol. This document supersedes the April 1, 1992, version.

MVSAM0122.01 Supplemental Assay Method for the Titration of Porcine Rotavirus Antibody (Constant Virus-Varying Serum Method). This SAM was rewritten to meet the current NVSL/CVB QA requirements, to clarify practices currently in use in the CVB-L, and to provide additional detail. No significant changes were made from the previous protocol. This document supersedes the April 1, 1992, version.

MVSAM0124.01 Supplemental Assay Method for Conducting the Hemagglutination Inhibition Assay for Equine Influenza Antibody. This document supersedes the October 28, 1994, version. This SAM was rewritten to meet the current NVSL/CVB QA requirements, to clarify practices currently in use in the CVB-L, and to provide additional detail. The following is a list of the significant changes made from the previous protocol:

8.1 Meet the requirements stated in 9 CFR, Part 113.217

8.2 Added a Reference Serum Positive Control

8.3 Removed the requirement for male chickens as the source of chicken RBC

8.4 Added the ability to store guinea pig serum (GPS) frozen until tested

8.5 Added GPS Autoagglutination Control

MVSAM0306.02 Supplemental Assay Method for the Titration of Feline Calicivirus in Cell Culture. This document supersedes the December 16, 1998, version.

The following is a list of the changes made from the previous protocol:

1. Changes have been made to the 2X Medium and Overlay Medium formulations.

2. 7.5% Sodium Bicarbonate has been included for preparation of the Overlay Medium.

3. A method was added to convert data from plaque forming units (PFU) to $\log_{10}$ 50% tissue culture infective doses ($TCID_{50}$).

4. Minor changes in terminology have been made and additional details have been added for clarification and consistency with other SAMs.

MVSAM0307.02 Supplemental Assay Method for the Titration of Feline Rhinotracheitis Virus in Cell Culture. This document supersedes the December 16, 1998, version.

The following is a list of the changes made from the previous protocol:

1. Changes have been made to the 2X Medium and Overlay Medium formulations.

2. 7.5% Sodium Bicarbonate has been included for preparation of the Overlay Medium.

3. A method has been added to convert the data from PFU to TCID.

4. Minor changes in terminology have been made and additional details have been added for clarification and consistency with other SAMs.

MVSAM0319.01 Supplemental Assay Method for Titration of Feline *Chlamydia psittaci* in Cell Culture. This is the revised version of the first draft written on July 27, 1995. This document was rewritten to meet the current NVSL/CVB QA requirements and to clarify practices currently in use in the CVB-L for testing live feline *Chlamydia psittaci* vaccines. Significant changes made from the previous draft protocol include:

8.1 Addition of 1M HEPES buffer to the maintenance medium to prevent rapid pH changes during the incubation of the test.

8.2 The final concentration of cycloheximide in the maintenance medium has been increased to 3 µg/ml.

8.3 The centrifugation of the inoculated McCoy Plates for enhancing chlamydia-host cell interaction was decreased to 539 x g, and is now performed immediately after inoculation of the McCoy Plates.

8.4 The incubation of the test has been extended to 132 ± 12 hr.

8.5 The methanol-ethanol fixative mixture was replaced by 80% acetone fixative.

8.6 The chlamydia titers are expressed as $\log_{10}$ 50% fluorescent antibody infective dose ($FAID_{50}$) instead of tissue culture infective dose ($TCID_{50}$).

MVSAM0320.01 Supplemental Assay Method for Titration of Feline *Chlamydia psittaci* in Embryonated Chicken Eggs. This document was rewritten to meet the current NVSL/CVB QA requirements, to clarify practices currently in use at the CVB-L for testing live *Chlamydia psittaci* vaccines (feline pneumonitis), and to reflect the change in Chlamydia Diluent in the procedure from the superseded protocol. This is the revised version of the first draft written on July 28, 1995.

These SAMs are also available as Adobe Acrobat pdf files on the world wide web (WWW) at http://www.aphis.usda.gov/vs/cvb/lab.

For those firms and interested parties with E-mail addresses and WWW access to the SAMs, this notification has been sent via electronic mail. If you would prefer to receive information from the Center for Veterinary Biologics via electronic mail, please send your E-mail address to cvb@usda.gov.

/s/ Randall L. Levings

Randall L. Levings
Director
Center for Veterinary Biologics